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Follicle Deviation in Ovulatory Follicular Waves with One or Two Dominant Follicles in Mares

JC Jacob[1,2], EL Gastal[3], MO Gastal[1], GR Carvalho[2], MA Beg[3] and OJ Ginther[1,3]

[1]*Eutheria Foundation, Cross Plains, WI, USA;* [2]*Animal Science Department, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil;* [3]*Pathobiological Sciences, University of Wisconsin, Madison, WI, USA*

Contents

The follicle and hormone aspects of diameter deviation and development of one dominant (≥28 mm) follicle (1DF) vs two dominant follicles (2DF) were studied in 32 ovulatory follicular waves in mares. Follicles were ranked each day as F1 (largest) to F3. The beginning of deviation was designated day 0 and preceded the first increase in the differences in diameter between F1 and F2 in the 1DF group and between a combination of F1 and F2 vs F3 in the 2DF group. One dominant follicle and 2DF developed in 21 (66%) and 11 (34%) waves, respectively. Double ovulations occurred in only one of the waves with 2DF. In 8/11 waves with 2DF, a second deviation occurred between F1 and F2 on 2.5 ± 0.4 days after the first deviation. On day 0, 1DF and 2DF waves were similar in number of days after ovulation, number of follicles, difference in diameter between F1 and F2, and plasma concentrations of LH, estradiol and immunoreactive inhibin. The interval from maximum FSH concentration to day 0 was longer ($p < 0.05$) and FSH concentration was lower ($p < 0.05$) on days -1 to 4 in the 2DF group. The similarities on day 0 in the characteristics of 1DF and 2DF waves despite the differences in the declining portions of the FSH profile indicated that a specific day of the FSH decline or a specific concentration were not factors in initiating deviation. Unlike reported results in heifers, the results in mares did not indicate a hormonal basis for the development of 2DF or two deviations.

Introduction

Follicle selection in monovular animals (e.g. mares, cows, women) is the process wherein usually only one follicle develops from a wave of growing follicles and continues to grow and ovulates. The eminent selection event during a follicular wave is diameter deviation and is retrospectively identifiable by continued growth of the developing dominant follicle (DF) and the beginning of reduced growth and regression of the remaining follicles (subordinates; Ginther et al. 1997). The characteristics and the intrafollicular and systemic hormonal events associated with the beginning of deviation have been reviewed for mares (Ginther 2000; Ginther et al. 2004a) and mares and heifers (Ginther et al. 2001, 2003; Beg and Ginther 2006) and have been compared between mares and women (Ginther et al. 2004b, 2005a). Briefly, dramatic changes in the insulin-like growth factor (IGF) system lead to increased free IGF1 in the most developed follicle before the beginning of diameter deviation and play a crucial role in the events that lead to deviation in both horses and cattle. Estradiol and LH receptors also play a role, at least in cattle. The intrafollicular events prepare the selected follicle for the decreasing availability of FSH from the wave-stimulating FSH surge and increasing availability of LH. Other follicles of the wave have a capability for future dominance similar to that of the largest follicle but do not have adequate time to attain the required preparatory stage. In this regard, the essence of deviation is a close two-way functional coupling between FSH and products of the follicles (inhibin, estradiol), so that the establishment of deviation or the destiny of the future dominant and subordinate follicles occurs in heifers in < 8 h.

In a direct comparative study of ovulatory follicular waves between mares and women, deviation began when the largest follicle and second-largest follicle were 22.7 and 20.3 mm in mares and 10.3 and 9.0 mm in women (Ginther et al. 2004b). The relative diameter of the largest follicle between the two species was similar at discernible events throughout the follicular wave. Thus, the follicle was 2.1 or 2.2 times larger in mares than in women at the peak of the wave-stimulating FSH surge (13.0/6.0 mm), at the beginning of deviation (22.7/10.3 mm) and at maximum diameter of the pre-ovulatory follicle (44.8/21.8 mm). Other similarities in the ovulatory wave between mares and women include (i) emergence of the future DF before the future largest subordinate follicle, (ii) length of intervals between sequential emergence of follicles of the wave, (iii) percentage increases in follicle diameter during the common growth phase preceding deviation, (iv) incidence of major anovulatory waves during the interovulatory interval, (v) incidence of pre-deviation follicles that reach maximum diameter an average of 1 day before the true deviation and (vi) occurrence of deviation during the FSH decline approximately 3 days after the peak of the FSH surge (Ginther et al. 2004b, 2005a). The remarkable similarities between mares and women throughout the ovulatory follicular wave encourage the use of mares as a comparative research model for study of the mechanisms of follicle deviation.

The occurrence of multiple (usually two) DF per wave reflects concentrations of circulating gonadotropins that differ from the changing concentrations for a single DF in heifers (Kulick et al. 2001; Beg et al. 2003; Acosta et al. 2005) and cows (Lopez et al. 2005). Therefore, a wave with 2DF is a natural model for studying the mechanisms of deviation. A DF has been defined as one that attains at least 28, 10, 10 and 13 mm in mares (Ginther 1993), heifers (Kulick et al. 2001), cows (Lopez et al. 2005) and women (Ginther et al. 2004b), respectively. Two dominant follicles in heifers may involve a single deviation or two separate deviations (Kulick et al. 2001; Beg et al. 2003; Acosta et al. 2005). Two

deviations are characterized by a first deviation involving the two largest follicles (future codominant follicles) vs the third largest follicle, followed an average of 2 days later by a second deviation between the two largest follicles. The phenomenon of two deviations has been reported only for heifers and apparently has not been studied in mares, cows and women. The ovulatory outcome of 2DF in cattle was not determined; all studies were performed during the first anovulatory wave. In pony mares, 2DF during the ovulatory wave may result in double ovulations, but usually only one follicle ovulates and the other follicle regresses after attaining the diameter of a DF (Ginther et al. 2004b).

The purpose of the present study was to determine the incidence and characteristics of 2DF with one or two deviations during the ovulatory follicular wave in mares. Changes in follicle diameters and systemic concentrations of FSH, LH, estradiol and immunoreactive (ir) inhibin were compared among waves with one vs 2DF, and results were interpreted in terms of the hormonal aspects of deviation.

Materials and Methods

Animals

Mares were handled according to the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching. The mares were mixed breeds of large ponies and apparent pony–horse crosses weighing 250–400 kg and aged 5 to ≥17 years. A total of 24 mares with a docile temperament and no apparent abnormalities of the reproductive tract, as determined by ultrasound examinations (Ginther 1995), was used in two consecutive oestrous cycles during April-August (northern hemisphere). The mares were kept under natural light in an open shelter and outdoor paddock and were maintained on alfalfa/grass hay with access to water and trace-mineralized salt. All mares remained healthy and in good body condition throughout the study. These same mares were used in a study of the temporal relationships among follicles and hormones during the periovulatory period and the repeatability of follicle diameters and hormone concentrations within individual mares (Jacob et al. 2007).

Ultrasonography and end points

B-mode ultrasonographic examinations were carried out daily for detection of ovulation and to determine diameter of follicles. The experiment started 15 days after an ovulation and encompassed three subsequent ovulations or two interovulatory intervals. The experimental period extended from 4 days before the first ovulation to 4 days after the third ovulation, but only the data temporally involving deviations were used in this study. A real-time ultrasound scanner with a linear array 7.5 MHz transducer was used for transrectal examination of the ovaries. Diameters of follicles ≥15 mm were measured (average of height and width) with the electronic calipers, and diameters of all other follicles (≥5 to <15 mm) were estimated. The diameters of the three largest follicles on each day were recorded without regard to day-to-day identity and were defined as F1 (largest) to F3. A DF was defined as one that attained ≥28 mm (Ginther 1993).

Diameter deviation in each follicular wave was based on retrospective examination of the diameter data profiles of individual follicles. Waves were removed from the analyses when the day of deviation was not apparent, owing to only one follicle per wave or obscurement by either intermingling follicles of a previous wave (Ginther et al. 2004a) or pre-deviation follicles within the ovulatory wave (Ginther et al. 2004b). In the remaining waves, the beginning of deviation was identified by the day preceding the first apparent change in the differences in diameter between follicles (Ginther et al. 1997). Days of deviation were established before other follicle and hormone data were inspected or analyzed. For waves with 1DF, deviation was detected by comparing the diameter growth profiles of F1 with the profile of F2. For waves with 2DF, deviation was detected by comparing the combined diameter profiles of F1 and F2 with the profile of F3. For 2DF, the subsequent profiles of F1 and F2 were examined for a second deviation. The single deviation for waves with 1DF and a single or first deviation for waves with 2DF were both defined as deviation 1, and the day of occurrence was designated day 0. For waves with 2DF and two deviations, the second deviation was defined as deviation 2, and its position in the wave was based on the number of days after deviation 1.

The ovulatory waves were divided into a group with 1DF and a group with 2DF. Sequential end points for comparisons among the two groups were diameter of F1, F2 and F3 on days -1 to 4; number of follicles 5–9, 10–14, 15–19 and ≥20 mm on days -1 and 0; concentrations of FSH, LH and estradiol on days -4 to 4; and concentrations of ir-inhibin on days -2, 0 and 2. Concentrations of ir-inhibin were assessed only on days -2, 0 and 2, owing to a limited availability of antigen. Single-point characteristics for the day of deviation 1 were number of days from the preceding ovulation and number of days to the next ovulation; diameters of F1, F2 and F3; concentrations of FSH, LH, estradiol and ir-inhibin; subsequent growth rate of F1 (days 0–4); day and concentration of maximum FSH; and number of days between maximum FSH and deviation 1. Follicular waves with 2DF were categorized into those with one or two deviations for characterization purposes.

Blood samples and hormone assays

Daily jugular blood samples were collected into heparinized tubes. Blood samples were centrifuged ($1500 \times g$ for 10 min) and decanted, and the plasma was stored at −20°C until assay. Plasma samples were assayed for FSH and LH by radioimmunoassay and estradiol and ir-inhibin by commercial kits, as validated and described for mare plasma in our laboratory (LH and FSH, Donadeu and Ginther 2002; estradiol, Ginther et al. 2005a; ir-inhibin, Donadeu and Ginther 2001). The ir-inhibin antibody recognizes dimeric forms of inhibin as well as free alpha subunits (Roser et al. 1994). The intra- and interassay coefficients of variation (CV) and mean sensitivity, respectively, were 9.2%, 18.4% and

1.1 ng/ml for FSH; 7.8%, 8.3% and 0.2 ng/ml for LH; and 10.0%, 4.9% and 0.1 pg/ml for estradiol. For ir-inhibin, the intraassay CV was 12.1% and sensitivity was 2.8 ng/ml.

Statistical analyses

Data for follicular and hormonal end points that were not normally distributed, according to a Kolmogorov–Smirnov test, were transformed to natural logarithms. Sequential diameters of follicles and plasma concentrations of hormones were normalized to day 0 (day of deviation 1) and were analyzed to determine the main effects of group (1DF vs 2DF) and day and for their interaction. The SAS MIXED procedure with a REPEATED statement was used to account for the autocorrelation between sequential measurements (9.3 Version; SAS Institute Inc., Cary, NC, USA). If a significant effect of group or group-by-day interaction was detected, unpaired Student's *t*-tests were used to locate differences in means between groups. If a significant day effect was obtained, differences between means within a group were examined by paired Student's *t*-tests. Single-point data were analyzed by one-way ANOVA, and frequency data were analyzed by chi-square tests of independence. Data are given as the mean ± SEM, unless otherwise stated. A probability of $p \leq 0.05$ indicated that a difference was significant, and probabilities between $p > 0.05$ and ≤ 0.1 indicated that a difference approached significance.

Table 1. Mean (± SEM) for characteristics at the beginning of the first follicle deviation in ovulatory follicular waves with one or two dominant follicles (DF)

Characteristics involving the first deviation	Number of dominant follicles (≥28 mm)		
	One (1DF)	Two (2DF)	p-value
No. of ovulatory waves	21	11	–
Day of occurrence (No. of days)			
After ovulation	15.3 ± 0.5	16.0 ± 0.6	NS
To next ovulation	7.3 ± 0.3	7.4 ± 0.4	NS
Diameter (mm) of			
F1 (largest follicle)	22.7 ± 0.4	23.9 ± 0.4	$p < 0.02$
F2	21.7 ± 0.5	22.7 ± 0.3	$p < 0.08$
F3	20.3 ± 0.5	20.8 ± 0.5	NS
F1 - F2	1.0 ± 0.3	1.1 ± 0.5	NS
Growth rate (mm/day) of F1 on days 0–4	3.1 ± 0.2	3.4 ± 0.0	NS
No. of follicles (mm)			
5–9	8.9 ± 1.0	8.5 ± 1.4	NS
10–14	5.8 ± 0.7	5.0 ± 1.1	NS
15–19	3.4 ± 0.5	3.8 ± 0.9	NS
≥20	2.0 ± 0.3	2.5 ± 0.7	NS
Concentrations of			
FSH (ng/ml)	12.5 ± 0.8	9.1 ± 0.7	$p < 0.005$
LH (ng/ml)	1.9 ± 0.2	1.8 ± 0.3	NS
Estradiol (pg/ml)	0.9 ± 0.1	0.9 ± 0.1	NS
Immunoreactive inhibin (ng/ml)	21.0 ± 0.3	19.7 ± 0.6	NS
Maximum FSH[a]			
No. of days after ovulation	9.8 ± 0.7	8.7 ± 0.9	NS
No. of days to deviation	5.5 ± 0.6	7.3 ± 0.9	$p < 0.05$
Concentration (ng/ml)	25.7 ± 2.5	26.5 ± 3.3	NS

[a]Maximum concentration between ovulation and deviation.

Results

Of the 48 ovulatory waves in the 24 mares, 32 waves were used in the analyses. Waves were omitted for the following reasons: anovulation (n = 2); no detectable deviation, owing to inadequate number of follicles (n = 3); and obscured deviation (n = 11). The number of waves in the group with 1DF was 21 (66%) and in the group with 2DF was 11 (34%). The number of waves with one and two deviations in the 2DF group was three and eight, respectively. Double ovulation occurred in 1/32 waves (3%) from a wave with 2DF and one deviation.

Single-point data for day 0 (day of beginning of deviation 1) for the 1DF and 2DF groups are shown (Table 1). Length of intervals from ovulation to deviation 1 and from deviation 1 to ovulation; diameter of F3; growth rate of F1 between days 0 and 4; concentrations of LH, estradiol and ir-inhibin; and day and concentration of maximum FSH did not differ between groups. The number of follicles 5–9, 10–14, 15–19 and ≥20 mm was not different between the 1DF and 2DF groups on day -1 (data not shown) or on day 0 (Table 1). The diameter of F1 on day 0 was greater, diameter of F2 approached being greater, concentration of FSH was less and number of days from maximum FSH to deviation was greater in the 2DF group than in the 1DF group.

The main effect of day was significant for diameters of F1, F2 and F3 on days -1 to 4 (Fig. 1). The group effect (1DF vs 2DF) was significant for F1, F2 and F3, owing to greater diameters in the 2DF group. The interaction of group and day was significant for F2 and F3, owing to increasing diameter after day 0 for ≥2 days in the 2DF group, compared with decreasing diameter after day 1 in the 1DF group. For FSH, the main effects of group and day were significant (Fig. 2); concentrations were less on days -1 to 4 in the 2DF group. Concentrations of LH showed only a significant day effect. Averaged over the two groups, LH concentrations first increased ($p < 0.03$) between days -2 (1.5 ± 0.1) and -1 (1.7 ± 0.2). There were no significant effects for estradiol, except for the main effect of day. Combined over the two groups, estradiol concentrations first increased ($p < 0.001$) between days -4 (0.4 ± 0.05) and -3 (0.5 ± 0.06); concentrations increased between days -4 and -2 in 94% of the waves and in all waves by day 0. For concentrations of ir-inhibin on days -2, 0 and 2, only the effect of day was significant (data not shown). Concentrations of ir-inhibin increased between days -2 (18.4 ± 0.4 ng/ml) and 0 (21.0 ± 0.3 ng/ml) in the 1DF group ($p < 0.002$) and between days -2 (15.5 ± 0.5 ng/ml) and 0 (19.7 ± 0.6 ng/ml) in the 2DF group ($p < 0.01$). An increase between days 0 and 2 was not significant for either group.

Diameters of F1, F2 and F3 are shown for the 1DF group and for the waves in the 2DF group with two deviations (Fig. 3). For the 1DF group, the difference in diameter between F1 and F2 was less ($p < 0.0001$) for day 0 (1.0 ± 0.3 mm) than for day 1 (3.9 ± 0.5 mm). Differences in diameter for F2 and F3 between days 0 and 1 were not significant. For the waves with 2DF and two deviations (n = 8), the interval between deviations 1 and 2 was 2 days for five waves and 3, 3 and 4 days for

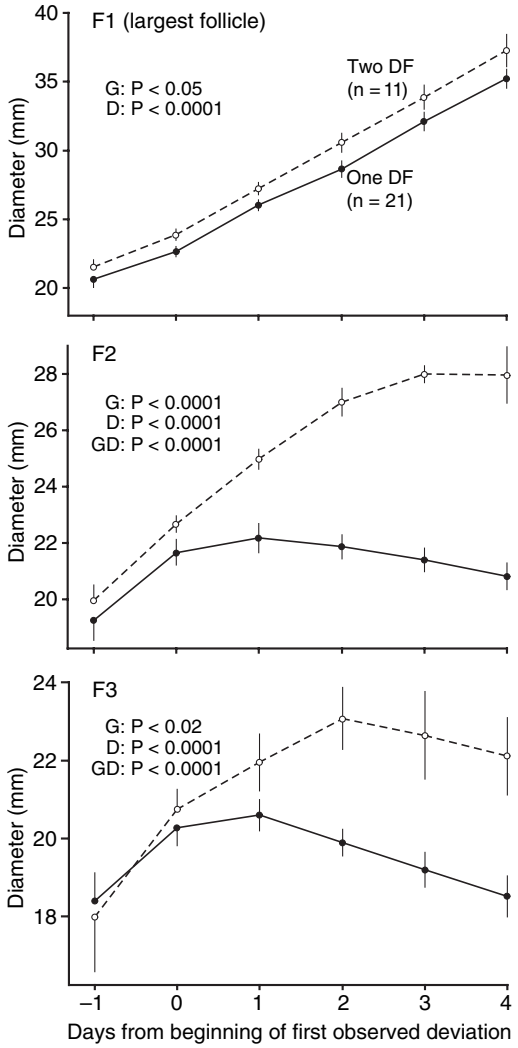

Fig. 1. Mean (±SEM) diameters of the three largest follicles (F1, F2 and F3) in ovulatory follicular waves with one vs two dominant follicles (DF). Significant probabilities for a main effect (G = group; D = day) and an interaction (GD) are shown

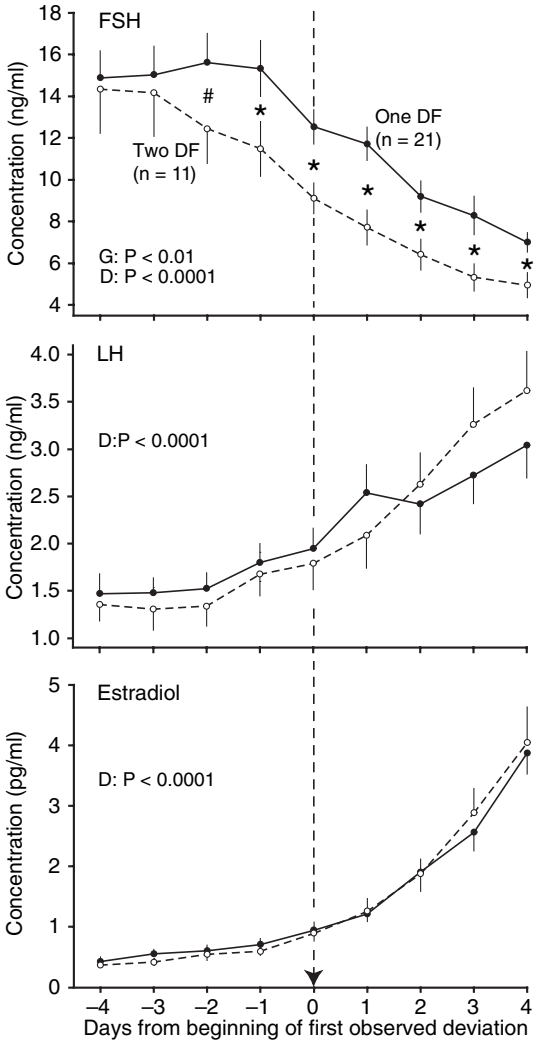

Fig. 2. Mean (±SEM) concentrations of plasma hormones for ovulatory waves with one and two dominant follicles (DF). Significant probabilities for a main effect (G = group; D = day) and an interaction (GD) are shown. An asterisk indicates days of a significant difference ($p < 0.05$) between groups, and a pound mark (#) indicates a difference that approached significance ($p < 0.07$)

the remaining waves (mean, 2.5 ± 0.4 days). The waves with 2 days between deviations were used to illustrate without interruption the characteristics of two deviations (Fig. 3). The difference between F1 and F2 for the five waves was not significantly different between days 0 (1.3 ± 0.2 mm) and 1 (1.6 ± 0.6 mm). The difference between F1 and F3 was less ($p < 0.002$) for day 0 (2.9 ± 1.4 mm) than for day 1 (5.3 ± 1.5 mm). For the second deviation, the difference in diameter between F1 and F2 was less ($p < 0.003$) for day 2 (1.0 ± 0.5 mm) than for day 3 (6.3 ± 1.3 mm); similar results were obtained for the remaining three waves when centralized to deviation (not shown). The difference in diameter between F1 and F2 showed a group-by-day interaction ($p < 0.006$) in the comparison of the 1DF group (one deviation) and the eight waves in the 2DF group that had two deviations (Fig. 3); the interaction represented significant differences that began on day 1 ($p < 0.02$) and continued thereafter.

Maximum diameter of the follicle that reached dominant status (based on diameter) and then regressed in the 2DF group was 30.8 ± 0.7 mm. Maximum diameter was attained 2.8 ± 0.3 days after deviation 1 or 19 days after ovulation and 4 days before the next ovulation. The diameter of the pre-ovulatory follicle on the day before ovulation in waves with only one ovulation approached significance ($p < 0.08$) between waves with 1DF (40.3 ± 0.9 mm) and waves with 2DF (42.7 ± 1.4 mm).

Discussion

This is the first study of follicle deviation that used waves with 2DF as a separate experimental group in mares. Although similar studies have been performed in cattle, the study is relevant because of the profound differences between species in the hormonal events temporally associated with 2DF and two deviations. The results are also relevant on a species comparison basis, owing to the striking similarities between mares

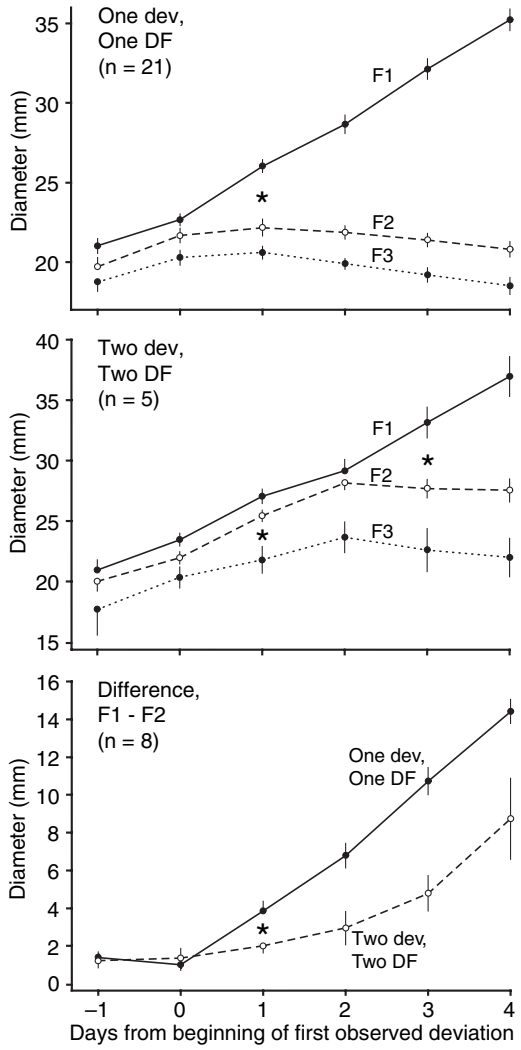

Fig. 3. Mean (±SEM) diameters of F1 (largest follicle), F2 and F3 for ovulatory waves with one or two dominant follicles (DF) and one or two deviations (dev). Upper panel: All mares with one dominant follicle and one deviation. Middle panel: Illustration of the relationships between two deviations, using only the mares with an interval of 2 days between deviations. Three additional mares had intervals of 3, 4 and 4 days (not shown). Lower panel: A comparison of the difference between the two groups in diameter of F1 - F2 for all waves in the two groups. In the upper two panels, an asterisk indicates the day after the beginning of a deviation or the first day when the difference in diameter between the two indicated follicles is greater ($p < 0.05$) than for the previous day. In the lower panel, the asterisk indicates the first day of a significant difference ($p < 0.05$) between groups

and women in relative diameter of the follicles among events throughout the ovulatory follicular wave and in the temporal relationships between deviation and the profile of the wave-stimulating FSH surge (see Introduction). The experiment was dependent on the reliability of assigning the day of the beginning of deviation by inspection of graphs that showed the changes in diameters of individual follicles. The process of deviation as observed during inspection of data has been substantiated mathematically by agreement between inspection and the results of a segmented linear regression approach in heifers (Bergfelt et al. 2003) and on a limited basis in mares (Ginther et al. 2003). In the present study, the follicles were ranked by diameter each day without regard to day-to-day identity (Ginther et al. 2007). This approach was used to minimize the length of the transrectal examinations. Ovulatory waves were not used for 11 of 48 (23%) waves when the day of the beginning of deviation was obscured by overlapping regressing follicles. An approximately similar incidence of obscurement by overlapping follicles has been reported (Ginther 2000; Ginther et al. 2004b). Overlapping of follicles preceding the beginning of deviation (common growth phase) also accounts for delaying the study of F1, F2 and F3 until day -1.

The occurrence of two deviations during the ovulatory wave in most mares with 2DF (incidence 8/11) has not been reported previously. A similar phenomenon has been reported for heifers during the first anovulatory wave (Beg et al. 2003; Acosta et al. 2005). The interval of 2 days between the first and second deviation in five of the eight waves was useful in illustrating the mean changes in diameters for F1, F2 and F3 in association with each deviation (Fig. 3). Between deviations 1 and 2, F1 and F2 increased in diameter in parallel as indicated by similar diameter differences. In the group with 2DF, the rate of growth of F3 began to decrease at deviation 1, but the subsequent diameters were greater than for F3 in the group with 1DF. This result indicated that 2DF were accompanied by greater diameter attainment of F3 although none of the F3 follicles reached the diameter of a DF. As expected, the first significant increase in the mean difference between follicles in the eight mares with two deviations and 2DF was on the day after the beginning of observed deviation for both deviations 1 and 2.

The number of days from ovulation to the first deviation (deviation 1; day 0) did not differ between mares with one or 2DF, as previously reported for heifers (Acosta et al. 2005) and cows (Lopez et al. 2005). The species differed in that F1 was a mean of 1.2 mm larger on the day of deviation 1 in waves with 2DF than in waves with 1DF in mares, but there were no differences in heifers for F1, F2 or F3. The difference in diameter between F1 and F2 on day 0 was similar between groups within each species and cannot be considered a factor in the development of one vs 2DF. In summary, the only characteristics of the follicle population detected on the day before and on the day of the beginning of deviation that were associated with development of the 2DF during equine ovulatory waves was a slightly greater diameter of F1 and a tendency (approached significance) for a greater diameter of F2. In contrast, the number of follicles ≥4 mm but not the diameters of F1 and F2 was greater in heifers that developed 2DF in the first anovulatory follicular wave (Kulick et al. 2001; Acosta et al. 2005).

Concentrations of FSH began to decrease before the beginning of deviation in the groups with one and 2DF, but the decrease began 2 days earlier in the group with 2DF. The earlier beginning of the decrease led to the longer interval from maximum FSH to deviation and apparently to the lower concentrations on days -1 to 4 in the group with 2DF. The earlier decrease in FSH relative to deviation is not attributable to the occurrence of deviation earlier in the oestrous cycle; the intervals from ovulation to deviation 1 and from deviation 1 to

the next ovulation were not different between the two groups. The lower FSH concentrations before the beginning of deviation in waves that later developed 2DF in mares contrasts with greater FSH concentrations 1 day before deviation in waves that developed 2DF in heifers (Kulick et al. 2001; Acosta et al. 2005) and cows (Lopez et al. 2005). Thus, higher FSH concentrations before deviation, as well as more follicles, is associated with the production of more waves with 2DF in cattle, but neither characteristic accounted for waves with 2DF in mares.

The dependence of deviation upon decreasing FSH has been well demonstrated by many temporal and FSH-manipulative studies in the three monovular species (see reviews cited in the Introduction). The occurrence of deviation in mares during the FSH decline in both groups but with an earlier beginning of the decline in the group with 2DF indicates that the FSH role in the initiation of deviation is a decrease in concentrations without dependence on the length of time from the beginning of the decline or the actual FSH concentration at the time of initiation. Apparently, therefore, other factors, as well as the FSH decline, contribute to the initiation of deviation (potential examples: stage of follicle development, increasing LH concentrations).

The lower FSH concentration after deviation in waves with 2DF in the present study in mares has been reported for heifers (Kulick et al. 2001; Beg et al. 2003; Acosta et al. 2005) and a similar tendency has been reported for cows (Lopez et al. 2005). The reduced post-deviation concentrations in FSH in waves with 2DF may account for the occurrence of a second deviation in 73% of waves with 2DF, as suggested for heifers (Kulick et al. 2001). However, this hypothesis has not been tested in either species.

Immunoreactive inhibin increased during the FSH decrease associated with deviation, as previously reported (Bergfelt et al. 2001; Ginther et al. 2005b). However, the increase was similar in the groups with 1DF and 2DF, despite the lower levels of FSH in the group with 2DF. In addition, estradiol concentrations were not different between the two groups during the days FSH was lower in the group with 2DF. Thus, the two hormones associated with FSH depression in mares (ir-inhibin and estradiol; Ginther et al. 2004a) did not account for the lower concentrations of FSH in the waves with 2DF. The present study involved total inhibin, and the role of inhibins in deviation will not likely be clarified until assays are available that distinguish between ir and bioactive forms. It is noteworthy, however, that the assay system that did not implicate ir-inhibin in the low FSH concentrations in mares with 2DF is the same system that has indicated consistently a negative relationship between the two hormones in several previous studies in mares (Bergfelt et al. 1991, 2001; Donadeu and Ginther 2001, 2004; Ginther et al. 2005b).

The pre-deviation increase in both plasma estradiol and LH in mares confirms previous findings (Ginther et al. 2004a). Concentrations of LH were not different between groups with 1DF and 2DF before deviation and at the beginning of deviation. This contrasts with findings in heifers (Kulick et al. 2001; Acosta et al. 2005) and cows (Lopez et al. 2005) of higher LH concentrations the day before the beginning of deviation in anovulatory waves with 2DF. There were no differences between the groups with 1DF and 2DF in post-deviation LH concentrations in the equine ovulatory follicular waves. Similarly, LH concentrations did not differ in cattle during the first 2 days after the beginning of deviation (Kulick et al. 2001; Acosta et al. 2005; Lopez et al. 2005). Estradiol did not differ between the two groups. In cows with 2DF, estradiol, as well as LH, increased post-deviation (Lopez et al. 2005).

In follicular waves with 2DF, double ovulations would originate from waves that have only one deviation, considering that a second deviation would involve regression of one of the two follicles. In the present study, only one wave produced double ovulations and was one of the three waves with 2DF and one deviation. One double ovulation in 32 waves (3%) is consistent with reported low double ovulation rates (2–3%) in ponies (Ginther 1992). Equine breeds and types vary widely in double ovulation rates (example: Thoroughbreds, 25%; Standardbreds, 15%) and is influenced by other factors (example: lactating and non-lactating Quarter Horses, 7% and 14%, respectively; Ginther 1992). The high double ovulation rate in some horse breeds when compared with ponies could reflect a greater rate of double DF or a greater rate of conversion of double DF to double ovulations. However, comparisons of the rate of double DF among breeds are not available.

Diameter of follicles that produce double ovulations are smaller on the day before ovulation than for single ovulations (Ginther 1992). In the present series, pre-ovulatory diameters of the one set of double-ovulating follicles were smaller (33.5 and 33.9 mm) than for all except one of the other 31 ovulations. The tendency (approached significance) for a larger pre-ovulatory follicle on the day before ovulation in waves with 2DF, but only one ovulation, than in waves with 1DF was unexpected and will require confirmation. The presence of an anovulatory DF that reached a maximum mean diameter of 31 mm in an average of 4 days before ovulation did not diminish the diameter of the other follicles on the day before it ovulated. This observation indicates that the reduced diameter of a pre-ovulatory follicle in waves with double ovulations occurred after the time the anovulatory DF in other waves reached maximum diameter.

In conclusion, the study produced the first information on the incidence of 2DF (≥28 mm; 11 of 32 waves) and two deviations (8 of 11 of the waves with 2DF) for ovulatory follicular waves in mares. The first deviation occurred on a similar day as the deviation in waves with 1DF and was between a combination of F1 and F2 vs F3. The second deviation occurred on a mean of 2.5 days later and was between F1 and F2. The only follicle difference preceding or at the beginning of deviation between the groups with 1DF and 2DF was a slightly larger F1 and a tendency for a larger F2 in the group that developed 2DF. No differences were found between waves that developed 1DF vs 2DF in LH, estradiol and ir-inhibin concentrations during 2 days before to 2 days after the beginning of deviation. Concentrations of FSH were lower in waves with 2DF

before, during and after the beginning of deviation, and the FSH decline began earlier. These results are important in that they indicate that the mechanisms underlying 2DF and two deviations in mares are different from those reported for heifers. Previous studies have documented the necessity of a decrease in FSH concentrations for the initiation of deviation. The earlier decline in FSH and the lower concentrations at the beginning of deviation in the group with 2DF suggests that the length of time that FSH has been declining and the attainment of a specific concentration are not requisite components of the FSH decline for the initiation of deviation.

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Author's address (for correspondence): OJ Ginther, Pathobiological Sciences, 1656 Linden Drive, University of Wisconsin, Madison, WI 53706, USA. E-mail: ginther@svm.vetmed.wisc.edu